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Increased bradykinin and "normal" angiotensin peptide levels in diabetic Sprague-Dawley and transgenic (mRen-2)27 rats

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Increased bradykinin and "normal" angiotensin peptide levels in diabetic Sprague Dawley and transgenic (mRen-2)27 rats.

Background. The transgenic (mRen-2)27 rat (TGR) is a high tissue renin, high angiotensin (Ang) II model of hypertension. When administered streptozotocin (STZ), TGRs develop a rapidly progressive diabetic nephropathy with renal failure over 12 weeks. Bradykinin (BK) and Ang II are potent vasoactive peptides that may participate in the vascular and metabolic abnormalities of diabetes.

Methods. TGR and Sprague-Dawley (SD) rats were administered STZ (diabetic) or citrate buffer (nondiabetic) at six weeks of age. Diabetic rats received daily ultralente insulin to maintain moderate hyperglycemia (~18 mM). Rats were sacrificed four- and eight-weeks post-STZ or vehicle.

Results. Diabetes did not modify the blood pressure of either SD rats or TGRs. Diabetes increased levels of BK-(1-9) and its metabolite BK-(1-7) in kidney, aorta, and heart of both SD rats and TGRs. Diabetes did not influence Ang II levels in plasma, kidney, aorta, heart, or adrenal gland of SD rats, but reduced to normal the elevated Ang II levels in plasma, kidney, aorta, and adrenal gland of TGRs.

Conclusions. STZ-induced diabetes was associated with elevated tissue levels of BK-(1-9) and "normal" circulating and tissue levels of Ang II. The increased BK-(1-9) levels were consistent with the participation of this peptide in the vascular and metabolic abnormalities of diabetes. However, the rapidly progressive nephropathy of diabetic TGRs was not associated with BK-(1-9) and Ang II levels in target organs that differed from those of diabetic SD rats.

Diabetes evokes a complex series of events that results in structural and functional abnormalities in many tissues, including the kidney and the cardiovascular system. Glomerular hyperfiltration, increased glomerular plasma flow, and an elevated glomerular capillary hydraulic pressure of early insulin-dependent diabetes in humans and experimental animals [1–7] suggest altered humoral control of microvascular function. Several lines of evidence suggest a role for bradykinin (BK) and angiotensin (Ang) peptides in the pathogenesis of diabetic lesions. BK is a potent endothelium-dependent vasodilator that increases vascular permeability and plays a primary role in inflammation [8], whereas Ang II has many actions, including vasoconstriction, causing an increase in intraglomerular capillary pressure [9] and stimulation of cytokine production and vascular hypertrophy [10].

Genetic evidence for a role of BK and Ang peptides in diabetic renal disease includes reports that polymorphisms of the angiotensin converting enzyme (ACE) and angiotensinogen genes contribute to the development of renal complications in insulin-dependent diabetes mellitus (IDDM) in humans [11, 12]. Pharmacological evidence for a role for BK and Ang II in diabetic vascular disease and nephropathy includes the reduction of vascular hypertrophy, the improvement of endothelial function, and the renoprotective effects of ACE inhibitors in both subjects with diabetes [13–15] and animal models of diabetic nephropathy [16–18]. Additionally, antagonists of the type 1 Ang II (AT1) receptor have renoprotective and antihypertrophic actions in diabetes [17, 18].

We investigated the hypothesis that Ang and BK peptide levels are altered in diabetes by measuring circulating levels of Ang peptides and tissue levels of Ang and BK peptides in rats with streptozotocin (STZ)-induced diabetes. We studied both Sprague-Dawley (SD) and transgenic (mRen-2)27 rats (TGRs). TGRs are SD rats transgenic for the mouse Ren-2 gene. This is a high tissue renin, high Ang II model of hypertension [19]. When administered STZ, TGRs develop a rapidly progressive diabetic nephropathy [20]. An aim of this study was to determine whether the accelerated glomerulosclerosis of TGRs is associated with increased renal Ang II levels. Albuminuria increases progressively over 12 weeks in diabetic TGRs, and the glomerular filtration rate (GFR) is increased at four and eight weeks and then subse-

Key words: hypertension, prorenin, angiotensin converting enzyme, endopeptidase, diabetic nephropathy.

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quently declines [20]. The most florid renal lesions occur after 12 weeks of diabetes in TGRs, with no morphological changes evident at four weeks and only basement membrane thickening evident at eight weeks (unpublished data from this laboratory) [20]. In the current study, rats were studied after four and eight weeks of diabetes, because this represents the time of development of the renal lesion in diabetic TGRs.

METHODS

Animals

Diabetes was induced in six-week-old female SD rats and heterozygous TGRs by the administration of 55 mg/kg STZ (Sigma, St. Louis, MO, USA) diluted in 0.1 M citrate buffer, pH 4.5, by tail vein injection following an overnight fast. Nondiabetic rats were injected with citrate buffer alone. All rats were allowed free access to tap water and standard rat chow containing 0.25% sodium and 0.76% potassium (GR2; Clarke-King & Co., Gladesville, NSW, Australia). Each week, rats were weighed and blood glucose (control, 4 to 8 mM; diabetic, more than 18 mM) was estimated using an AMES glucometer (Bayer Diagnostics, Melbourne, Victoria, Australia). Systolic blood pressure was recorded in conscious rats by tail cuff plethysmography (model PE-300 programmed electrosphygmanometer; Narco Bio-Systems, Inc., Austin, TX, USA). Diabetic rats received daily intraperitoneal injections of insulin (two to four units; Ultratard, Novo Nordisk, Denmark). Experimental procedures adhered to the guidelines of the National Health and Medical Research Council of Australia's Code for the Care and Use of Animals for Scientific Purposes and were approved by the Bioethics Committee of the University of Melbourne.

At four- and eight-weeks post-STZ or vehicle, rats were killed by decapitation. Trunk blood was collected for the measurement of plasma levels of renin, angiotensinogen, ACE, neutral endopeptidase 24.11 (NEP), and Ang peptides, and the left kidney, adrenal glands, heart (cardiac ventricles) and aorta were rapidly removed, weighed, and immediately homogenized in 4 M guanidine thiocyanate, 1% trifluoroacetic acid in water (GTC/TFA) for the measurement of tissue levels of Ang and BK peptides.

Angiotensin and bradykinin peptide assay

Plasma levels of Ang II and its biologically inactive precursor Ang I were measured as described previously [21]. Briefly, trunk blood (2 to 3 ml) was rapidly collected into tubes containing 0.5 ml inhibitor solution (1 mM renin inhibitor acetyl-His-Pro-Phe-Val-Sta-Leu-Phe-NH_2 [22], 146 μM pepstatin, 50 mM 1,10-phenanthroline, 125 mM ethylenediaminetetraacetic acid (EDTA), 2 g/liter neomycin sulfate, 2% dimethyl sulfoxide, and 2% ethanol in water) at 4°C. The blood was centrifuged, and the plasma (1 to 2 ml) was immediately extracted with Sep-Pak C_{18} cartridges (Waters Chromatography Division, Milford, MA, USA). Ang peptides were acetylated and piperidine treated before high-performance liquid chromatography (HPLC) and assay of HPLC fractions by *N*-terminal–directed radioimmunoassay (RIA) [21, 23]. Data were corrected for recovery as reported elsewhere [21].

Tissues homogenized in GTC/TFA were processed as described previously [24] before acetylation and piperidine treatment and HPLC and measurement of Ang and BK peptides by *N*-terminal–directed RIA [21, 24]. Data were corrected for recovery as reported elsewhere [21, 24].

Renin, prorenin, angiotensinogen, angiotensin converting enzyme, and neutral endopeptidase 24.11 assays

Trunk blood for the measurement of renin, prorenin, angiotensinogen, ACE, and NEP was collected into heparinized tubes on ice and then centrifuged, and the plasma was rapidly frozen on dry ice and stored at −80°C. The plasma concentrations of active renin, prorenin, and angiotensinogen were measured as described previously [19, 25]. Renin and prorenin activities were expressed as Goldblatt units per milliliter, using hog renin as reference standard (National Standards Laboratory, Holly Hill, London, UK). ACE enzymatic activity was measured as described by Friedland and Silverstein [26], and NEP enzymatic activity was measured as described by Yandle et al [27].

Statistics

Data are presented as means ± SEM. Peptide ratios were calculated for individual rats, and the ratios are also presented as means ± SEM. Data were analyzed by unpaired *t*-test and by analysis of variance. The effects of diabetes were analyzed at four- and eight-weeks post-STZ in SD rats and TGRs separately. In addition, TGRs were compared with SD rats of the same age. Logarithmic transformation of the data was performed when required to obtain similar variances between groups. Differences were considered significant at a P of less than 0.05. Statistical analyses were performed using SuperANOVA® (Abacus Concepts, Inc., Berkeley, CA, USA).

RESULTS

Blood pressure, body weight, heart weight, and kidney weight

Nondiabetic TGRs were hypertensive and had cardiac hypertrophy (Table 1). Nondiabetic TGRs had a lower body weight than nondiabetic SD rats at four weeks but not at eight weeks postvehicle. Additionally, nondiabetic TGRs had higher kidney weight/body weight ratios than nondiabetic SD rats at four weeks (Table 1). Diabetes had no effect on the blood pressure of either SD rats or

Table 1. Blood pressure, body weight, and tissue weights of non-diabetic and diabetic SD rats and TGR

	Duration of diabetes *weeks*			
	4		8	
Parameter	SD	TGR	SD	TGR
Systolic blood pressure *mm Hg*				
Non-diabetic	118 ± 6	150 ± 2[d]	108 ± 2	152 ± 6[d]
Diabetic	106 ± 2	155 ± 4[d]	116 ± 4	151 ± 4[d]
Body weight *g*				
Non-diabetic	298 ± 5	266 ± 5[d]	283 ± 11	275 ± 14
Diabetic	247 ± 8[b]	229 ± 14[a]	222 ± 11[b]	213 ± 7[b]
Heart weight *mg*				
Non-diabetic	860 ± 23	899 ± 22	848 ± 18	1064 ± 46[d]
Diabetic	758 ± 40[a]	764 ± 68	751 ± 26[b]	781 ± 30[b]
Heart weight/body weight ratio *mg/g*				
Non-diabetic	2.88 ± 0.04	3.40 ± 0.10[d]	3.03 ± 0.13	3.88 ± 0.12[d]
Diabetic	3.07 ± 0.14	3.31 ± 0.13	3.44 ± 0.18	3.70 ± 0.17
Left kidney weight *mg*				
Non-diabetic	1097 ± 22	1089 ± 18	1041 ± 21	1033 ± 47
Diabetic	1257 ± 32[b]	1457 ± 76[b,c]	1565 ± 59[b]	1504 ± 55[b]
Left kidney weight/body weight ratio *mg/g*				
Non-diabetic	3.68 ± 0.05	4.11 ± 0.10[d]	3.72 ± 0.15	3.77 ± 0.10
Diabetic	5.16 ± 0.24[b]	6.62 ± 0.51[b,c]	7.14 ± 0.31[b]	7.17 ± 0.41[b]

Values are expressed as means ± SEM; $N = 6$ to 10. Abbreviations are: Sprague Dawley (SD), transgenic m(Ren-2)27 rat (TGR).
[a] $P < 0.05$, [b] $P < 0.01$, in comparison with respective non-diabetic group of the same duration
[c] $P < 0.05$, [d] $P < 0.01$, in comparison with respective SD rats of the same duration

TGRs. In comparison with the corresponding nondiabetic rats, diabetic rats had a reduced body weight. There were no differences between body weights of diabetic SD rats and TGRs, and the heart weights and heart weight/body weight ratios of diabetic SD rats and TGRs were similar (Table 1). Diabetic SD rats and TGRs had higher kidney weights and kidney weight/body weight ratios than the corresponding nondiabetic rats. Both the kidney weight and the kidney weight/body weight ratio were increased in diabetic TGRs compared with diabetic SD rats at four weeks but not at eight weeks (Table 1).

Plasma glucose, renin, prorenin, angiotensinogen, angiotensin converting enzyme, and neutral endopeptidase 24.11

Plasma glucose levels were similar for SD rats and TGRs and were approximately threefold higher in diabetic animals (Table 2). Plasma renin levels were 1.6- to 4-fold higher in TGRs than in SD rats for both nondiabetic rats at four and eight weeks and for diabetic rats at eight weeks. Prorenin levels were 37- to 61-fold higher in both nondiabetic and diabetic TGRs than in SD rats, with a corresponding increase in the prorenin/total renin ratio in TGRs (Table 2). Diabetes was without effect on renin and prorenin levels at four weeks. At eight weeks, prorenin levels were 2.9-fold higher in diabetic than nondiabetic SD rats and 2.6-fold higher in diabetic than nondiabetic TGRs. However, diabetes did not affect the prorenin/total renin ratio. Plasma angiotensinogen levels were similar in SD rats and TGRs, except at eight weeks, when angiotensinogen levels were lower in diabetic TGRs than in diabetic SD rats (Table 2). Diabetic rats had lower plasma angiotensinogen levels than nondiabetic rats at eight weeks in SD rats and at both four and eight weeks in TGRs. Nondiabetic SD rats and TGRs had similar plasma ACE levels at four weeks, but at eight weeks, plasma ACE levels of nondiabetic TGRs were lower than those of nondiabetic SD rats (Table 2). Diabetes increased plasma ACE levels at four weeks in SD rats and at eight weeks in TGRs. Thus, at four weeks, plasma ACE levels of diabetic SD rats were higher than those of diabetic TGRs, whereas at eight weeks, plasma ACE levels of diabetic TGRs were higher than those of diabetic SD rats (Table 2). SD rats and TGRs did not differ with respect to plasma NEP levels, and diabetes had no effect on these levels (Table 2).

Plasma angiotensin peptides

In agreement with their increased renin levels, nondiabetic TGRs had higher plasma Ang II and Ang I levels than nondiabetic SD rats at both four and eight weeks (Fig. 1). The Ang II/Ang I ratio provides an index of the rate of conversion of Ang I to Ang II. Nondiabetic TGRs had a lower Ang II/Ang I ratio than nondiabetic SD rats at four and eight weeks, in agreement with the lower plasma ACE levels in nondiabetic TGRs than in nondiabetic SD rats at eight weeks (Table 2). Diabetes had no effect on plasma Ang peptide levels in SD rats, but reduced Ang II and Ang I levels at four weeks and Ang II levels at eight weeks in TGRs. Thus, the plasma Ang peptide levels of diabetic TGRs were similar to those of nondiabetic and diabetic SD rats. In contrast

Table 2. Plasma levels of glucose, renin, prorenin, angiotensinogen, ACE, and NEP of non-diabetic and diabetic SD rats and TGR

	Duration of diabetes *weeks*			
	4		8	
Parameter	SD	TGR	SD	TGR
Plasma glucose *mM*				
Non-diabetic	5.6 ± 0.4	5.7 ± 0.5	ND	ND
Diabetic	15.0 ± 0.9[b]	17.0 ± 0.9[b]	18.9 ± 1.0	18.1 ± 1.1
Plasma renin *mGU/ml*				
Non-diabetic	0.10 ± 0.01	0.16 ± 0.01[d]	0.09 ± 0.01	0.36 ± 0.18[c]
Diabetic	0.19 ± 0.05	0.15 ± 0.03	0.19 ± 0.06	0.62 ± 0.17[d]
Plasma prorenin *mGU/ml*				
Non-diabetic	0.15 ± 0.01	5.6 ± 0.4[d]	0.15 ± 0.02	9.2 ± 0.2[d]
Diabetic	0.14 ± 0.02	6.3 ± 0.9[d]	0.43 ± 0.04[b]	24.0 ± 3.2[b,d]
Plasma prorenin/total renin ratio %				
Non-diabetic	62 ± 3	97 ± 1[d]	61 ± 6	97 ± 2[d]
Diabetic	50 ± 8	97 ± 1[d]	73 ± 4	98 ± 1[d]
Plasma angiotensinogen *pmol/ml*				
Non-diabetic	407 ± 13	407 ± 16	467 ± 23	456 ± 24
Diabetic	397 ± 24	335 ± 29[a]	337 ± 19[b]	286 ± 15[b,c]
Plasma ACE *nmol/ml/min*				
Non-diabetic	80 ± 4	77 ± 5	79 ± 3	64 ± 4[c]
Diabetic	105 ± 5[b]	65 ± 6[d]	84 ± 5	115 ± 7[b,d]
Plasma NEP *nmol/ml/min*				
Non-diabetic	0.21 ± 0.01	0.25 ± 0.02	0.21 ± 0.02	0.23 ± 0.01
Diabetic	0.23 ± 0.01	0.27 ± 0.04	0.24 ± 0.01	0.25 ± 0.01

Values are expressed as means ± SEM; $N = 6$ to 10 Abbreviations are: Sprague Dawley (SD), transgenic m(Ren-2)27 rat (TGR), Goldblatt unit (GU), angiotensin converting enzyme (ACE), neural endopeptidase (NEP).
[a] $P < 0.05$, [b] $P < 0.01$, in comparison with respective non-diabetic group of the same duration
[c] $P < 0.05$, [d] $P < 0.01$, in comparison with respective SD rats of the same duration

to the increased plasma ACE levels of diabetic rats, diabetes did not modify the plasma Ang II/Ang I ratio of either SD rats or TGRs.

Tissue angiotensin peptides

In agreement with the increased plasma renin and Ang peptide levels in TGRs, Ang II levels were higher in kidney, adrenal gland, and aorta of nondiabetic TGRs than in nondiabetic SD rats (Fig. 2 and Table 3). However, Ang II levels were not elevated in the heart of TGRs (Table 3). The increased renal Ang II levels in nondiabetic TGRs were associated with a reduced Ang I level and an increased Ang II/Ang I ratio at eight weeks (Fig. 2).

Similar to the effects seen in plasma, diabetes did not modify tissue Ang II levels in SD rats, but reduced Ang II levels in kidney and adrenal gland at four and eight weeks and in aorta at four weeks in TGRs (Fig. 2 and Table 3). Thus, diabetes tended to "normalize" the increased tissue Ang II levels of TGRs. Diabetes did not modify Ang II levels in heart (Table 3). Diabetes reduced Ang I levels in kidney of SD rats at eight weeks (Fig. 2). For adrenal gland, aorta, and heart, Ang I levels were close to or below the limit of detection (adrenal gland, <50 fmol/g; aorta, <20 fmol/g; heart, <2 fmol/g). The reduction of kidney Ang II levels of TGR by diabetes was associated with a decrease in the Ang II/Ang I ratio in this tissue at both four and eight weeks (Fig. 2).

Tissue bradykinin peptides

A comparison of the levels of BK-(1-9) and its inactive metabolite BK-(1-7) in tissues of SD rats and TGRs showed lower BK-(1-7) levels in kidney, adrenal gland, and heart, and lower BK-(1-9) levels in heart of TGRs at eight weeks (Fig. 3 and Table 4). The BK-(1-7)/BK-(1-9) ratio provides an index of the rate of conversion of BK-(1-9) to BK-(1-7). The BK-(1-7)/BK-(1-9) ratios of TGRs were less than those of SD rats in kidney and aorta at four and eight weeks and in adrenal gland at eight weeks, consistent with reduced metabolism of BK-(1-9) to BK-(1-7) in these tissues of TGRs (Fig. 3 and Table 4).

Diabetes increased BK peptide levels in kidney, aorta, and heart, but not in adrenal gland (Fig. 3 and Table 4). For kidney, diabetes increased both BK-(1-7) and BK-(1-9) levels at four weeks but not at eight weeks (Fig. 3). These increases were not statistically significant when SD rats and TGRs were analyzed separately, but achieved statistical significance when SD rats and TGRs were analyzed together by two-way analysis of variance [BK-(1-7): $F_{1,36} = 6.151$, $P = 0.018$; BK-(1-9): $F_{1,36} = 6.553$, $P = 0.015$]. For aorta, diabetes increased BK-(1-7) levels in both SD rats and TGRs and BK-(1-9) levels in TGRs at eight weeks (Table 4). For heart, diabetes increased BK-(1-7) levels in SD rats and TGRs at both four and eight weeks and BK-(1-9) levels in SD rats and TGRs at four weeks and SD rats at eight weeks (Table 4). Diabetes did not influence the BK-(1-7)/BK-(1-9) ratio, except for an in-

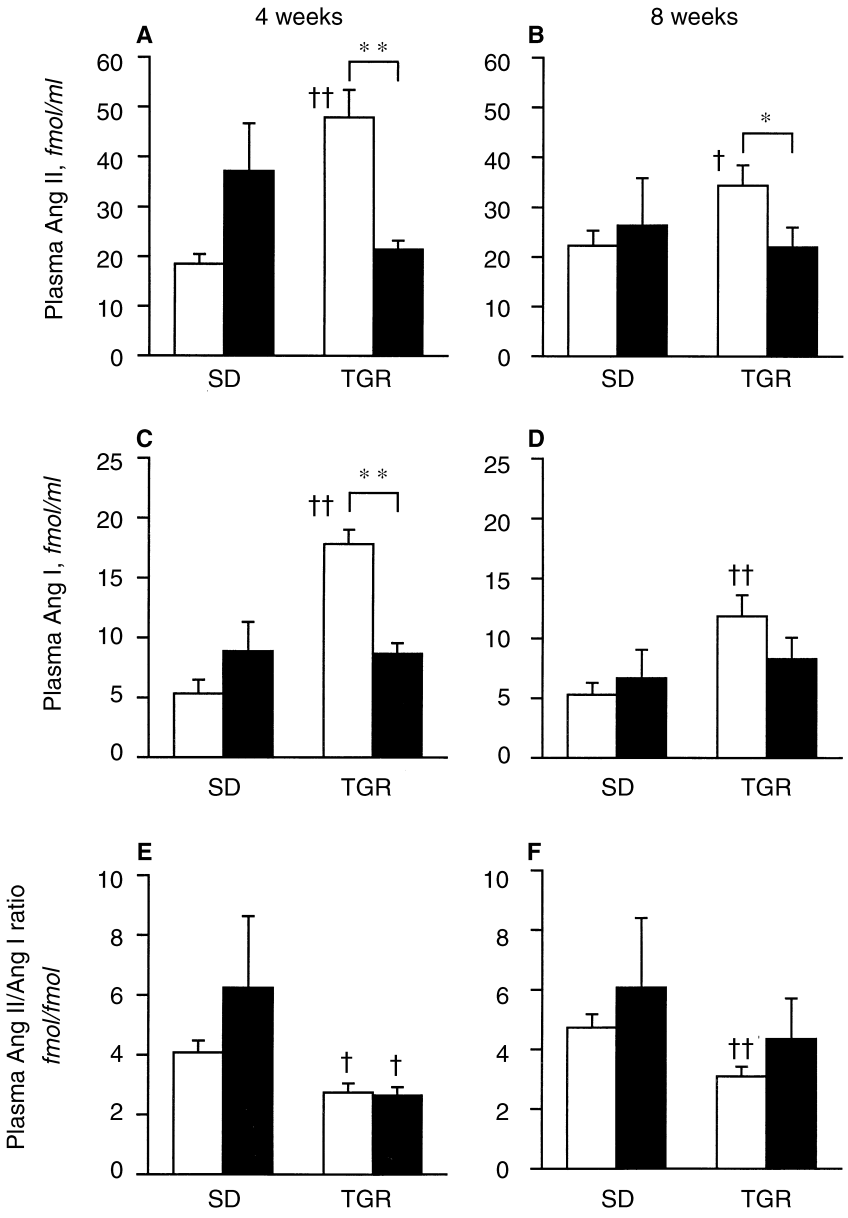

Fig. 1. Plasma levels of angiotensin (Ang) II (*A*, *B*) and Ang I (*C*, *D*), and plasma Ang II/Ang I ratio (*E*, *F*) in nondiabetic (□) and diabetic (■) Sprague Dawley (SD) rats and transgenic (mRen-2)27 (TGR) rats at four- and eight-weeks post-streptozotocin (STZ) or vehicle. Values are expressed as means ± SEM ($N = 10$); *$P <$ 0.05, **$P < 0.01$ diabetic vs. corresponding nondiabetic rats; †$P < 0.05$, ††$P < 0.01$ TGRs vs. corresponding SD rats.

crease in BK-(1-7)/BK-(1-9) ratio in kidney of SD rats at eight weeks (Fig. 3).

DISCUSSION

The main findings of this study were the increased tissue BK-(1-7) and BK-(1-9) levels in diabetic SD rats and TGRs, whereas Ang II levels were similar in nondiabetic and diabetic SD rats, and diabetes reduced the elevated Ang II levels of nondiabetic TGRs to the levels of nondiabetic and diabetic SD rats. The parallel increases in levels of BK-(1-9) and its metabolite BK-(1-7), without a decrease in the BK-(1-7)/BK-(1-9) ratio, were consistent with increased kinin formation caused by increased kallikrein activity in diabetes, rather than to reduced kinin metabolism by ACE or NEP. Further evidence that the increased BK-(1-9) levels were not consequent to reduced metabolism was the failure of diabetes to reduce plasma levels of NEP and ACE. Our finding of increased BK-(1-9) levels in diabetes is in agreement with previous reports of increased renal tissue kallikrein levels and urinary kallikrein and kinin excretion in diabetic rats and humans treated chronically with insulin [28–30].

The early years of diabetes are characterized by increased flow and pressure in the glomerular, retinal, and cutaneous capillary circulations [31]. Consistent with a generalized vasodilation is the increased blood volume relative to body weight in STZ diabetic rats [5]. Kinins

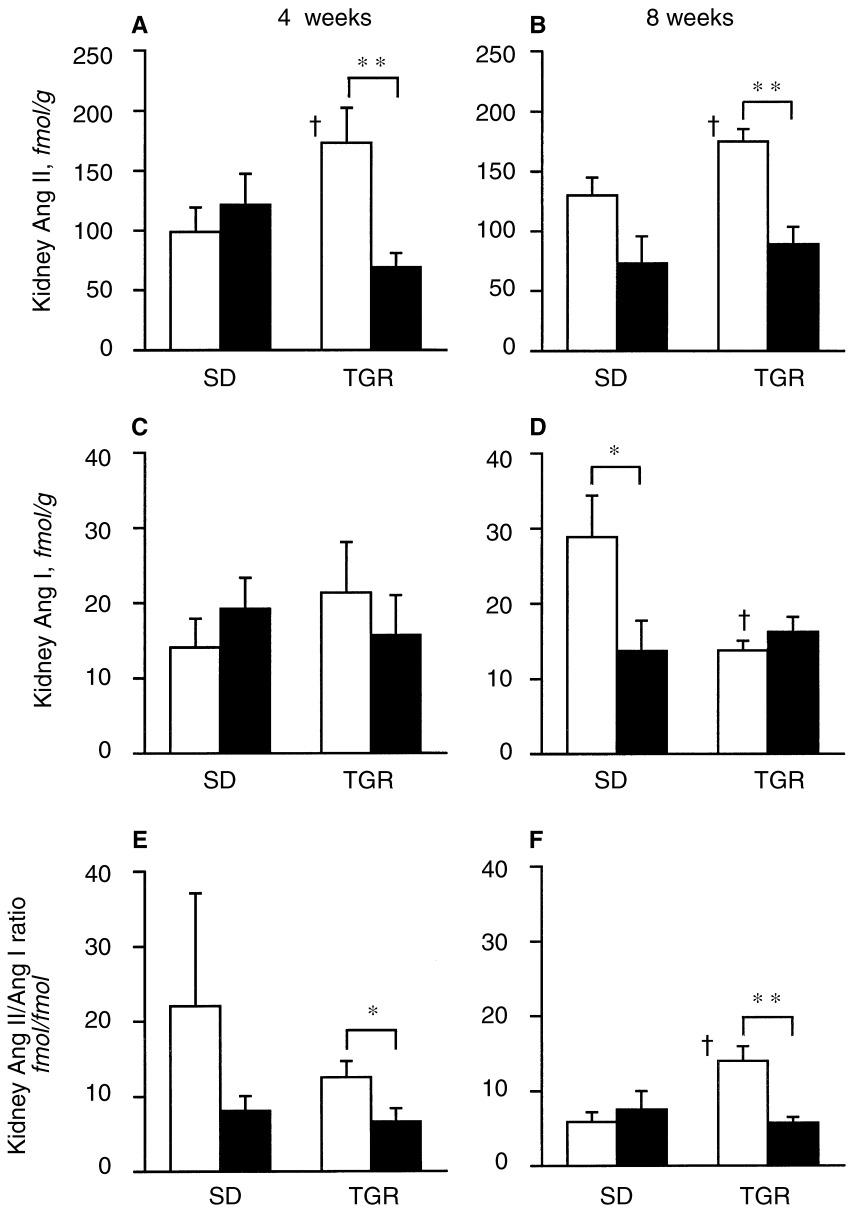

Fig. 2. Kidney levels of Ang II (*A*, *B*) and Ang I (*C*, *D*), and kidney Ang II/Ang I ratio (*E*, *F*) in nondiabetic (□) and diabetic (■) SD rats and TGRs at four- and eight-weeks post-STZ or vehicle. Values are expressed as means ± SEM ($N = 10$); $^*P < 0.05$, $^{**}P < 0.01$ diabetic vs. corresponding nondiabetic rats; †$P < 0.05$ TGRs vs. corresponding SD rats.

are potent endothelium-dependent vasodilators that stimulate an endothelial release of nitric oxide and prostaglandins [8]. If this finding of increased BK-(1-9) levels in kidney, aorta, and heart represents a generalized phenomenon, it suggests an important role for BK-(1-9) in the vasodilation of diabetes and the pathogenesis of diabetic vascular disease.

A state of glomerular hyperfiltration has been consistently demonstrated in patients with newly diagnosed IDDM [1–4], as well as in early stages of experimental diabetes in animals [5–7, 32]. In the latter, this hyperfiltration was shown to result from concomitant elevations in glomerular plasma flow and mean glomerular capillary hydraulic pressure [5–7, 32]. These early glomerular hemodynamic abnormalities are implicated in the pathogenesis of the glomerulopathy that eventually develops in this disorder [16], although there is uncertainty concerning the role of glomerular hyperfiltration in the development of nephropathy in humans [33]. The increased microvascular flow is due to reduced precapillary and postcapillary resistance, whereas the increased capillary pressure implies a relative increase in postcapillary resistance [31]. Increased kinin levels may contribute to the increased microvascular flow, and the relative increase in postcapillary resistance may be due to an increased renovascular sensitivity to Ang II in diabetes [34, 35]. Consistent with this suggested role for Ang II in modulating postcapillary resistance, AT1 receptor antagonism

Table 3. Ang II levels in adrenal, aorta, and heart of non-diabetic and diabetic SD rats and TGR

	Duration of diabetes *weeks*			
	4		8	
Parameter	SD	TGR	SD	TGR
Adrenal Ang II *fmol/g*				
Non-diabetic	1316 ± 180	2298 ± 142[d]	1116 ± 174	1648 ± 139[c]
Diabetic	1405 ± 238	816 ± 133[b,c]	745 ± 119	1208 ± 140[a,c]
Aorta Ang II *fmol/g*				
Non-diabetic	47 ± 9	106 ± 7[d]	23 ± 4	42 ± 7[c]
Diabetic	50 ± 10	61 ± 8[b]	33 ± 9	56 ± 15
Heart Ang II *fmol/g*				
Non-diabetic	9.6 ± 2.1	13.9 ± 2.7	10.1 ± 1.2	11.5 ± 2.1
Diabetic	9.0 ± 1.4	9.3 ± 1.9	8.1 ± 1.8	13.6 ± 2.4

Values are expressed as means ± SEM; $N = 9$ to 10. Abbreviations are: Sprague Dawley (SD), transgenic m(Ren-2)27 rat (TGR), angiotensin II (Ang II).
[a] $P < 0.05$, [b] $P < 0.01$, in comparison of diabetic and corresponding non-diabetic rats
[c] $P < 0.05$, [d] $P < 0.01$, in comparison with respective SD rats of the same duration

selectively normalized the elevated glomerular capillary hydraulic pressure and ultrafiltration coefficient in the diabetic kidney [36]. Other actions of BK-(1-9) that may contribute to diabetic renal disease include the stimulation of mesangial cell proliferation, an effect potentiated by insulin, and the stimulation of collagen production by mesangial cells, an effect inhibited by insulin [37].

Several studies addressed the role of kinins in diabetes by administering either the nonspecific kallikrein inhibitor aprotinin or antagonists of the type 2 BK-(1-9) (B2) receptor. The administration of aprotinin, or a first generation B2 receptor antagonist D-Arg,[Hyp^3,Thi^{5,8},D-Phe^7]-BK-(1-9), lowered renal blood flow and the GFR [28, 30, 38], suggesting a contribution by an altered renal kallikrein-kinin system to the development of glomerular hypertension [39]. However, there are conflicting reports of the effects of the second-generation B2 receptor antagonist HOE 140 in diabetes. An acute administration of HOE 140 had no effect on GFR, renal plasma flow, filtration fraction, or sodium excretion in STZ diabetic rats [38, 40], although acute HOE 140 administration did reduce GFR in ACE inhibitor-treated diabetic rats [38]. Moreover, Allen et al found no effect of 24-week HOE 140 administration on albuminuria or glomerular ultrastructure in STZ diabetic rats [17]. Similarly, Rumble, Komers, and Coopers found no effect of three-week HOE 140 administration on mesenteric vascular hypertrophy in STZ-diabetic rats [18]. By contrast, a chronic administration of HOE 140 reduced diuresis and proteinuria in STZ-diabetic mice, without effect on glycemia or body weight [41]. Responsiveness to B2 receptor antagonism may depend on the stage of diabetes, in that secondary structural changes of the vasculature may prevent response to B2 antagonism. Furthermore, hyperglycemia may blunt the response to increased kinin levels by contributing to defective endothelial nitric oxide production in diabetes [35, 42]. Moreover, kinins may act through the type 1 BK-(1-9) (B1) receptor, and their effects are thereby unaffected by B2 receptor antagonism. These data indicate a need to investigate the expression of B1 and B2 receptors in diabetes and the role of kinins in the initiation of diabetic microvascular disease.

The mechanisms of kinin generation are complex and may involve the participation of tissue kallikrein, plasma kallikrein, and kallikrein-independent mechanisms [43]. Moreover, the regulation of kallikrein activity is complex and may involve kallistatin [44] and other inhibitors and activators of kallikrein. Further experiments are required to elucidate the specific tissue compartments where kinin levels were increased and the mechanisms responsible for the increased BK levels observed in tissues of diabetic rats in this study.

Previous studies found either suppressed or normal plasma renin levels [10, 18], suppressed plasma angiotensinogen levels [45], and normal plasma Ang II levels in diabetes [32, 38]. Diabetes has variable effects on renal levels of renin, angiotensinogen, and ACE, although Ang II receptors are reduced in diabetic kidney [10, 45]. Anderson, Jung and Ingelfinger found increased levels of renin protein and renin and angiotensinogen mRNA and reduced ACE levels in diabetic kidney [36]. Immunocytochemical studies revealed that whereas proximal tubule ACE was reduced in diabetic kidney, ACE immunostaining was increased in glomeruli and renal vasculature [36, 46]. Kennefick et al and Vora et al reported normal renal Ang II levels in diabetic rats, in agreement with these findings [34, 38].

Both ACE inhibitors and AT1 receptor antagonists have renoprotective effects and reduce vascular hypertrophy in diabetes [14–18, 38, 40]. Although kinins are implicated in mediating some of these effects of ACE inhibitors [18, 38, 40], the renoprotective and antihypertrophic effects of AT1 receptor antagonists indicate that Ang II contributes to the vascular abnormalities of diabetes [17, 18]. The finding of normal renal Ang II levels in diabetic kidney does not exclude a role for this peptide

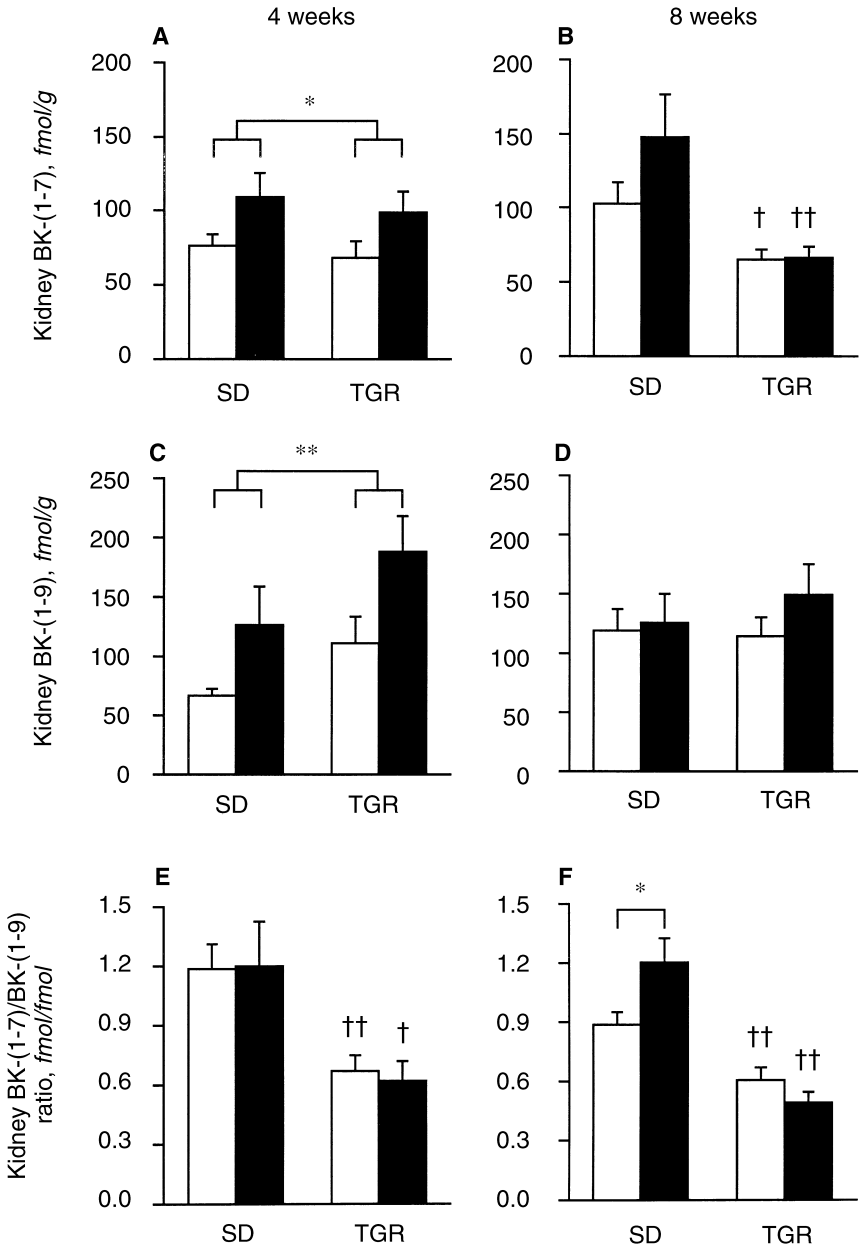

Fig. 3. Kidney levels of bradykinin (BK) BK-(1-7) (*A*, *B*) and BK-(1-9) (*C*, *D*), and kidney BK-(1-7)/BK-(1-9) ratio (*E*, *F*) in nondiabetic (□) and diabetic (■) SD rats and TGRs at four- and eight-weeks post-STZ or vehicle. Values are expressed as means ± SEM ($N = 10$); *$P < 0.05$, **$P < 0.01$ diabetic vs. corresponding nondiabetic rats; †$P < 0.05$, ††$P < 0.01$ TGRs vs. corresponding SD rats. For the BK-(1-7) and BK-(1-9) levels at four weeks, the differences between nondiabetic and diabetic rats were not statistically significant when SD rats and TGRs were analyzed separately, but were statistically significant when SD rats and TGRs were analyzed together by two-way ANOVA.

in diabetic renal disease. Diabetes is associated with increased renal sensitivity to Ang II in rats [34, 35] but not in humans [47]. The increased renovascular sensitivity to Ang II in diabetic rats, despite reduced renal Ang II receptor levels [10, 45], may be due, in part, to the inhibition by hyperglycemia of nitric oxide production by the renal vasculature [35]. In contrast to the reduction in Ang II receptor number in kidney, diabetes is associated with increased numbers of Ang II receptors in heart, adrenal gland, and liver [45].

Our finding of similar kinin levels in SD rats and TGRs and "normalization" of Ang II levels in diabetic TGR suggests that the accelerated glomerulopathy of diabetic TGR is not due to BK-(1-9) and Ang II levels that differ from those of diabetic SD rats. The cause of the accelerated glomerulosclerosis of diabetic TGRs remains unclear but may result from the additive effects of elevated levels of BK-(1-9), "normal" levels of Ang II, induction of other cytokine pathways by these two peptides, and the hemodynamic effects of the TGR phenotype. In separate studies, we showed that both the ACE inhibitor perindopril and the AT1 antagonist valsartan normalized blood pressure, prevented accelerated glomerulosclerosis, and attenuated the changes in renal function in diabetic TGRs (Kelly, Cooper, Skinner, Wilkinson-Berka, manuscript in preparation) [20], suggesting that "normal"

Table 4. BK-(1-7) and BK-(1-9) levels and BK-(1-7)/BK-(1-9) ratio in adrenal, aorta, and heart of non-diabetic and diabetic SD rats and TGR

	Duration of diabetes *weeks*			
	4		8	
Parameter	SD	TGR	SD	TGR
Adrenal BK-(1-7) *fmol/g*				
Non-diabetic	151 ± 32	248 ± 71	217 ± 31	119 ± 19[c]
Diabetic	150 ± 23	176 ± 39	323 ± 73	176 ± 34
Adrenal BK-(1-9) *fmol/g*				
Non-diabetic	146 ± 35	271 ± 71	202 ± 36	161 ± 30
Diabetic	106 ± 10	179 ± 43	235 ± 51	196 ± 32
Adrenal BK-(1-7)/BK-(1-9) ratio *fmol/fmol*				
Non-diabetic	1.15 ± 0.12	0.85 ± 0.13	1.12 ± 0.10	0.78 ± 0.10[d]
Diabetic	1.49 ± 0.21	1.04 ± 0.12	1.38 ± 0.11	0.85 ± 0.05[d]
Aorta BK-(1-7) *fmol/g*				
Non-diabetic	47 ± 8	46 ± 11	44 ± 10	28 ± 6
Diabetic	69 ± 17	62 ± 17	108 ± 25[a]	73 ± 17[b]
Aorta BK-(1-9) *fmol/g*				
Non-diabetic	33 ± 7	65 ± 16	40 ± 11	36 ± 9
Diabetic	47 ± 8	79 ± 24	62 ± 12	91 ± 14[b]
Aorta BK-(1-7)/BK-(1-9) ratio *fmol/fmol*				
Non-diabetic	2.11 ± 0.66	0.90 ± 0.20[c]	1.38 ± 0.26	1.11 ± 0.27
Diabetic	1.51 ± 0.25	0.82 ± 0.07[c]	1.96 ± 0.36	0.75 ± 0.08[d]
Heart BK-(1-7) *fmol/g*				
Non-diabetic	22 ± 4	18 ± 2	26 ± 3	17 ± 2[c]
Diabetic	41 ± 6[a]	34 ± 5[b]	62 ± 11[b]	34 ± 11[a,d]
Heart BK-(1-9) *fmol/g*				
Non-diabetic	23 ± 8	19 ± 3	32 ± 5	23 ± 4
Diabetic	69 ± 27[a]	59 ± 15[a]	84 ± 20[a]	31 ± 4[d]
Heart BK-(1-7)/BK-(1-9) ratio *fmol/fmol*				
Non-diabetic	1.29 ± 0.12	1.04 ± 0.12	1.01 ± 0.19	0.86 ± 0.09
Diabetic	0.91 ± 0.14	0.94 ± 0.33	0.86 ± 0.08	1.16 ± 0.27

Values are expressed as means ± SEM; $N = 10$. Abbreviations are: Sprague Dawley (SD), transgenic m(Ren-2)27 rat (TGR), bradykinin-(1-7) [BK-(1-7)], bradykinin-(1-9) [BK-(1-9)].

[a] $P < 0.05$, [b] $P < 0.01$, in comparison of diabetic and corresponding non-diabetic rats

[c] $P < 0.05$, [d] $P < 0.01$, in comparison with respective SD rats of the same duration

Ang II levels have a pathogenic role in diabetic renal disease in this model. Moreover, the renal Ang system is compartmentalized, and the "normal" renal Ang II levels measured in this and other studies may mask increased Ang II levels in specific renal compartments such as the renal vasculature and glomerular mesangium.

Diabetes reduced Ang II levels in plasma, kidney, adrenal gland, and aorta of TGRs without affecting plasma renin levels. The mechanism by which diabetes reduced Ang II levels in TGRs is uncertain, although it may have been due in part to the lower plasma angiotensinogen levels in diabetic TGRs. Plasma ACE levels were elevated in eight-week diabetic TGRs, but the Ang II/Ang I ratio was unaltered by diabetes, indicating normal conversion of circulating Ang I to Ang II in these rats. However, diabetic TGR kidney showed a reduced Ang II/Ang I ratio, suggesting that reduced renal ACE activity contributed to the lower renal Ang II levels in diabetic TGRs.

In conclusion, diabetes was associated with elevated tissue levels of BK-(1-9) and "normal" circulating and tissue levels of Ang II. The increased BK-(1-9) levels were evidence for the participation of this peptide in the vascular abnormalities of diabetes. However, the rapidly progressive nephropathy of diabetic TGR was not associated with BK-(1-9) and Ang II levels that differed from those of diabetic SD rats.

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APPENDIX

Abbreviations used in this article are: ACE, angiotensin converting enzyme; Ang, angiotensin; AT1 receptor, type 1 angiotensin II receptor; B1 receptor, type 1 bradykinin receptor; B2 receptor, type 2 bradykinin receptor; BK, bradykinin; GFR, glomerular filtration rate; GTC/TFA, 4 mol/liter guanidine thiocyanate, 1% trifluoroacetic acid in

water; HPLC, high-performance liquid chromatography; IDDM, insulin-dependent diabetes mellitus; NEP, neutral endopeptidase 24.11; RIA, radioimmunoassay; SD, Sprague-Dawley; STZ, streptozotocin; TGR (mRen-2)27 rats, Sprague Dawley rats transgenic for the mouse Ren-2 gene.

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